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Development of the Continuously Variable Volume Reactor for Flow Injection AnalysisDesign, Capabilities and Testing*

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Abstract

A new apparatus for mixing sample and reagent in flow injection analysis is described. The continuously variable volume reactor (CVVR) replaces the conventional mixing coil in a flow injection manifold to provide mixing and dilution. A linear actuator motor allows control of the chamber volume via LabVIEW software. The chamber volume can be incremented in steps of 1 µL over the range 68-1704 µL. In addition, the chamber has an integral variable-speed stirring unit that is also under computer control. Experiments were performed to evaluate the dispersion characteristics of this new device, evaluate the volume reproducibility, and understand the mixing characteristics. Use of the chamber is shown in the determination of iron(II) in pond water, and in NIST SRM 1643d with excellent results and a detection limit of 3.7 µg/L iron(II). Advantages of the CVVR and future research activities using the device are discussed.

Introduction

Since its introduction in 1975 [1] flow injection analysis (FIA) has been known for its capability to generate reproducible concentration gradients [2-9]. This has made FIA the flexible, widely applicable technique that it is. Typically, flow injection (FI) manifolds contain a mixing coil (10–300 cm) that is placed between the injection valve and the detector. The use of a mixing coil improves the axial mixing of the sample and the carrier (reagent) stream without significantly increasing the longitudinal mixing [10] and this can be further improved by the knotting of the tubing [11]. Given a constant flowrate of carrier (or reagent), the well-defined volume of the mixing coil, and reproducible mixing a flow injection (FI) manifold will produces a peak that is a highly reproducible gradient of the injected analyte concentration. With the current trend toward moving analysis out in the field [12], FIA and its sibling, sequential injection analysis (SIA), are obvious choices.

Both techniques can be applied to a wide variety of analytes (especially environmental important inorganic ions) and can be designed to be compact and minimize reagent consumption. However, when designing a portable or remote (unattended autonomous operation) FIA analyzer the instrument is typically limited in capability. This is too say, a manifold is constructed in the laboratory for the expected range of analyte concentrations, the general type of sample, and only a specific analyte.

Taking a closer look, the limiting factor in most cases is the size of the mixing coil. Of all the variables in a FI system, flowrate(s), injected volume, detection wavelength, and mixing coil volume the only one that has to remain constant in a remote device is the mixing coil volume. It is possible (with good design) to change out the mixing coil in a portable instrument but totally impractical in a remote instrument. Thus, a better alternative is needed.

The continuously variable volume chamber (€VVR) [13] was designed to fit this need. A variable-volume mixing chamber under computer control fits perfectly into the picture of a remote FIA or SIA instrument. With the addition of the CVVR all components of the system are directly under computer control and can be changed quickly and easily for each analyte without the need for the analyst to be present.

In this paper we introduce the CVVR, its design, capabilities, and discuss the possibilities for its use in FI. The determination of iron(II) in pond water and NIST SRM 1643d "Trace Elements in Water" is shown as an example of the use of the CVVR. Finally, we will discuss future developments of the CVVR and indicate research avenues we will be pursuing.

Background

The idea of using a mixing chamber rather than a mixing coil has been around almost since the invention of FIA. A full review of the use of mixing chambers (also called gradient chambers and reaction chambers) is not possible here, but readers are referred to the following web page for a full list of pertinent material [14]. A mixing chamber is placed into a flow injection manifold for one of a number of reasons namely, sample dilution, standard dilution, titration, extended range calibration, homogenous mixing, or matrix matching. Inherently a mixing chamber increases the dispersion of a peak due to the intimate mixing of a sample and carrier (or reagent) in (typically) a large volume. Dependent upon the geometry (and volume) of the chamber, more or less dispersion (widening) of the FI peak is obtained.

The tail of the FI peak produced in a gradient chamber has an exponential nature due to the prolonged washout of the chamber as new carrier comes in. It has been shown that with the correct design of such a chamber can produce an almost perfect exponential decay [15]. While this might be useful for electronic dilution [16-18], single peak calibration [8, 19-24], and zone sampling [16, 25-27], the general use of gradient chambers has not been adopted due to the width (time) of the FI peak produced and thus the decrease in throughput that results.

Situations where a gradient chamber is useful are where intimate mixing of sample and carrier/reagent cannot be reproducibly achieved using conventional mixing coils. Examples include highly viscous samples [28], non-aqueous samples [29], and samples with a high dissolved solid content [30]. The idea of using forced mixing (in the case of a gradient chamber using a small magnetic stirrer) may well be preferential in other circumstances, especially for kinetically limited reactions where the kinetics of mixing can be decoupled from the kinetics of reaction. Again though the inherent dilution that occurs limits sensitivity, and this can become the limiting factor in many situations.

The CVVR (Figure 1a) proposed in this paper is best described as a hybrid mixing device when thinking of both gradient chambers and mixing coils. In the situation where stirring is enabled it is an end on designed mixing chamber the volume of which can be changed to any value within the range 68-1704 µL. However, when stirring is disabled the chamber acts more like a conventional mixing coil. As the flow enters the chamber (Figure 1b) the linear velocity of the solution is reduced. This is due to the widening of the flow path from 0.80 mm i.d. (tubing) to 6.35 mm (chamber). Assuming no variation of the flowrate (i.e. due to pump pulsations) laminar flow conditions are not disturbed due to the conical design (45°) of the transition between these two dimensions, and the reverse transition at the outlet.

Experimental

The overall system for this work is shown in Figure 2. Computer control was

provided by a Power Macintosh 4400/200 (#M5767LL/A - Apple Computer Inc., Cupertino, CA USA *www.apple.com*), with a Crescendo G3 upgrade card (#B4G3-300-512 - Sonnet Technologies, Irvine, CA USA *www.sonnettech.com*). National Instruments (Austin, TX USA *www.ni.com*) LabVIEW 5.1.1 software (#776698-03) and PCI-1200 data acquisition card (#777386-01) were used send out control signals to the motors and injection valve, and receive feedback from the chamber location sensors (see below). A 350 MHz Pentium II Computer (Dell Computer Corporation, Round Rock TX USA – *www.dell.com*) was used to run Vision 3.32 software (Unicam Instruments now part of ThermSpectronics, Rochester NY USA *www.thermo.com*) to acquire absorbance readings from a Unicam UV4 Spectrophotometer.

Hardware and Electronics

The housing for the CVVR is shown in Figure 3. The linear stepper motor, rotary stepper motor, and motor drive cards were purchased from Haydon Switch and Instrument Inc., Waterbury CT, USA (*www.hsi-inc.com*). Initially, lower power motors were used however they were insufficient to reliably move the chamber piston or rotate the mixer. The final motors used were therefore #46341-12 (linear actuator), and #46440-12 (rotary). These motors were each controlled via two TTL lines from the LabVIEW software for enable/disable and forward/reverse movement. In addition, speed control was provided via 0-5 V square waves of appropriate frequency generated from the counter-timers on the PCI-1200 board.

Connections for the TTL signals were initially directly from the PCI-1200 board to the drive cards, however the current draw from the drive cards was found to be too high for the PCI-1200 board (resulting in blown pins) and so photovoltaic relays #PVA1054 (Newark Electronics, Chicago, IL USA *www.newark.com*) were placed in between to avoid this problem.
The two-position (low-pressure) six-port injection valve (#C22) and micro-electric actuator (#EHCA) were manufactured by Valco Instrument Co. (Houston, TX USA *www.valco.com*).

Switching between the load and inject positions was controlled via TTL logic from the PCI-1200 card. Two location sensors for the position (volume) of the chamber were linear potentiometers also purchased from Newark. A 5 V signal from the PCI-1200 board was place on one side of the potentiometer and the variable voltage difference was measured from the other side.

Power was supplied to the CVVR using an Elpac W7224-D5 power supply (Irvine, CA USA *www.elpac.com*). This voltage was distributed to the various electronics using voltage regulators (#P6SMB15AT3) from Newark. This was especially important for the motor drive cards that required a steady 13.5 V voltage. Other electronic components, such as resistors and wire were also purchased from Newark.

Design of the Manufacture of the Continuously Variable Volume Reactor

Manufacture of the CVVR was performed by PlasmaTech, Houston, TX USA (www.plantfloor.com/tx/plastechprecisionmachining.htm). The design of the CVVR was done completely in-house. Many considerations went into the design to make sure that the device would serve a wide range of needs. Table 1 shows some pertinent parameters of the CVVR. The chamber volume was designed to cover the normal range of volumes found in FI mixing coils. However, in designing the layout of the chamber it was felt very important not to introduce regions where solution could be stagnant. This would be most likely at the transition from the 0.8 mm i.d. tubing into the chamber and at the outlets and thus at each end the wall of the tube opens up at a 45° angle (Figure 1b). This describes a "cone" which maintains laminar flow patterns while decreasing the average linear velocity. When the chamber is fully closed these two cones are in

contact with each other and describe the minimum volume of the chamber - 68 µL (taking into account the mixer).

The chamber diameter of 6.35 mm (1/4") was considered to be a good compromise between making the chamber too small, and therefore needing a really long piston, and making it too large where the volume increments would be two big and where the linear velocity might be slowed too much. With this diameter and the minimum step size of 0.0254 mm (1/1000") the chamber volume can be adjusted in 0.82 µL increments.

The current design of the CVVR is the second prototype built in our laboratory. The first design had a no mixing device, only one inlet and one outlet, and was limited to only 1.5" of movement of the chamber piston. The mixing device was added due to problems associated with mixing when the chamber volume was changed as the sample bolus flowed through the chamber (this new approach to FIA will be discussed in a subsequent paper). Additionally, the mixer was felt necessary to improve the mixing when two streams entered the chamber (see the discussion section). Increasing the inlets to two obviously allows for a double line FI manifold to be built. Finally, addition of multiple outlets (in this work three) was deemed necessary to allow for multiple detection of analytes from one injection (split stream) and to allow for sensors to be incorporated into the chamber directly.

Reagents

All reagents were of analytical reagent grade quality and purchased from Fisher Scientific (Pittsburgh, PA USA *www.fishersci.com*). Milli-Q water (Millipore, Bedford, MA USA *www.millipore.com*) was used throughout to make up solutions.

Samples

Samples of pond water from the UNF campus where collected and analyzed within two days of collection. Immediately after collection the samples were filtered through a 0.45 µm cellulose acetate filter (#A04SP04700) and stored in 500 mL Nalgene bottles (#02-924-6E) in a refrigerator at 4°C until use. Portions of the pond water were spiked by addition of small volumes of a 1000 mg/L iron reference solution (#SI124-500).

A portion of NIST (Gaithersburg, MD USA www.nist.gov) SRM 1643d "Trace metals in water" was analyzed for total iron content straight out of the bottle. An additional 25 mL portion was analyzed after addition of 0.0978 g ascorbic acid (#A62), and 340 µL of 50.5%(w/v) NaOH (SS254). The base was necessary due to the pH of the SRM (in 0.5 M HNO_3) being too low for the reaction to proceed.

Procedures

Bromothymol blue (BTB) was used to study the dispersion of the system without chemical reaction. For the preparation of stock BTB, 0.500 g of solid was taken and dissolved in 400 mL, 16 mL of 0.1 M NaOH was added and the solution was made up to volume (1 L) with Milli-Q water (4.00×10^{-4} M BTB). The pH of this solution was ~10 maintaining the indicator in the blue form. This stock solution was then diluted 25 mL to 1 L in 5×10^{-4} M NaOH to give a BTB concentration of 2.00×10^{-5} M. This working solution was injected in a stream of 5×10^{-4} M NaOH so that there would be no possibility of loss of BTB absorption due to a pH gradient across the peak. The absorbance of this solution at 616 nm was approximately 0.80.

For the preparation of the iron standards, 40, 80, 120, 160, and 200 µL of the 1000 mg/L iron reference solution were added to 100 mL volume flasks and 0.40 g of ascorbic acid added to each to convert the iron(III) to iron(II). In the single line manifold this was injected in to a solution of 1.55×10^{-2} M o-phenanthroline (#AC15753) and 4 g/L ascorbic acid. This

was made up by adding 2.7933 g o-phenanthroline to 500 mL of Milli-Q water in a beaker, heating on a hotplate at 50°C until dissolved, transferring to a 1 L volumetric flask, adding an additional 400 mL of Milli-Q water, allowing the solution to come to room temperature, adding 4 g of ascorbic acid and then making up to the mark with Milli-Q water. This solution has a slight yellow color and is stable for two weeks. For the double line manifold the iron(II) was injected into a carrier stream of 4 g/L ascorbic acid, and this stream was then mixed in the CVVR with the o-phenanthroline/ascorbic acid solution prepared above. The ascorbic acid was added to all solutions to minimize refractive index effects.

Once the manifold for each experiment had been assembled for each set of experiments injections were made using a program written in-house in LabVIEW. This approach ensured that each injection was done in the same way and that enough BTB or iron(II) solution (being pumped though the loop) filled the loop completely between injections.

Each set of experiments comprised five replicate injections. For each run the flowrate of the carrier/reagent stream(s) was measured using a 10 mL volumetric flask and a stopwatch. Injections were initiated via the LabVIEW program and manually coordinated with the collection of the detector response (Vision software). Upon completion of the five injections, absorbance-time profiles were exported from the Vision software into ASCII x-y pair format and transferred to the Macintosh for processing in Excel.

Calibration of the CVVR chamber was performed by measuring the mass of water either drawn up by or dispensed from one of the outlets upon movement of the chamber (the pump was not on). Masses determined were corrected for the evaporation rate of water and converted to volumes using the density of water measured at temperature in a 10 mL volumetric flask.

Calibration of the injection loop volumes was achieved by injection of BTB solutions (described above) into 5 x 10^{-4} M NaOH and flowing this directly into a 10 or 25 mL volumetric flask. The injected BTB solution absorbance at 616 nm was determined. The volumetric flask was made up to the mark with 5 x 10^{-4} M NaOH, mixed, and measured at 616 nm. The dilution of the BTB solution and the number of injections made allowed determination of the volume of each loop.

Results and Discussion

The initial impetus for this work was to develop a replacement for a mixing coil that could be used in an unattended remote FI instrument. This meant the design needed to fulfill three criteria; 1) the chamber volume and mixer speed be completely under computer control, 2) the chamber volume have definition better than needed for typical FI experiments, and 3) have a range of volumes wide enough to accommodate >95% of the published FI manifolds. In the second version of the CVVR (described here) the linear motor and rotary motor are completely under computer control, volume definition is better than 2 µL (see volume calibration) and the volume range is from 68-1704 µL (13-339 cm of 0.8 mm i.d. Teflon tubing). Including the connection tubing from the valve to the CVVR and from the CVVR to the detector the volume range is from 251-1887 µL (38-374 cm).

After the first CVVR had been built it was realized that other criteria were important; detection of the actual location of the chamber (feedback), and the addition of a mixing device were needed. The inclusion of location sensors (linear potentiometers) was implemented to provide the feedback of the chambers location.

However, this approach does not have the distance (and thus volume) resolution that the linear motor can provide (0.0254 mm step). Therefore, a more accurate

alternative is currently being sought and the location sensors are only used to record the location of the chamber, not to provide feedback control, in this work.

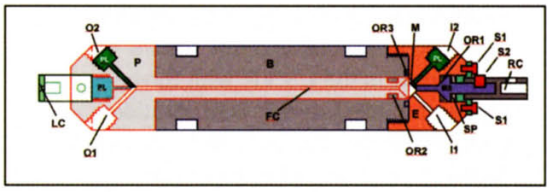
As can be seen by the design of the CVVR, the replacement of the conventional mixing coil with a computer controlled mixing chamber has resulted in a sophisticated piece of instrumentation. This comparison highlights the drawbacks of the CVVR namely; complexity, the need for precision machined components, the requirement for computer control and cost. One of the most difficult features to implement on the CVVR was the mixer. The balance between maintaining fluid integrity, and providing low resistance for the rotary motor to spin the mixer was not a trivial optimization. The key factor in this was the material of the o-ring (OR1 in Figure 1a) which was initially rubber but was replaced with Viton. The mixer was subsequently found to operate with no problems up to a speed of 240 steps per second. This corresponds to five revolutions per second, or 300 rpm.

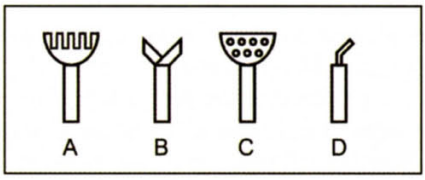
Chamber Volume Calibration and Reproducibility

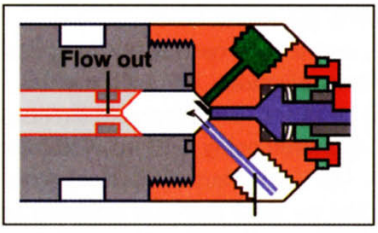
It was observed that the volume of the chamber changes linearly with distance and that these volumes agree excellently with the predicted volumes. There is a slight difference in the volume changes between opening and closing the chamber and this is likely due to the ease at which the motor can push or pull the piston. The reproducibility of the volumes improves with size, but even at small volumes this variation is less than 1%. These numbers can likely be improved by modifications to keep the linear motor screw from rotating, which results in no movement of the piston along the axis of travel.

Figure 1. The continously vairable volume reactor: Continusously variable volume reactor schematic: P = piston; B = body; E = end piece; FC = flow channel; LC = linear motor connector; RC = rotary motor connector; M = mixer; I1 and I2 = flow inglets; O1 and O2 = flow outlets; MS = mixer spindle; OR1, OR2 and OR3 = O rings; PL = plug; S1 and S2 = screws. Close up of flow into CVVR chamber Mixing device geometries (designs were chosen with design that would increase the radial and minimizing longitudinal mixing: A = "Hanukah"; B = "Y"; C = "Paddle"; D = "Tubing").

a.)







Other experiments have shown that the incremental volume reproducibility (from 0 to 6.35 mm, from 6.35 to 12.7 mm, etc.) is also excellent with a mean of 205±3 µL (1.2%) over the length 0-50.8 mm in both the opening and closing directions. Based on the nominal diameter of the chamber this should be 203 µL.

The reproducibility of the chamber was also look at in terms of the peak heights from the injection of BTB solutions. Five replicate injections were made at a chamber volume of 817 µL (25.4 mm open) for five successive runs.

For each set of injections the chamber was returned to the closed position and then opened back up to 817 µL. Over the 25 injections the peak height was 0.4606±0.0065 (1.40%) and a two way ANOVA showed that there was no significant difference between the within run and between run variances.

Figure 2. Instrumental layout. C = carrier (or reagent); P = pump; S = injection valve (sample); M = mixer; D = detector; W = waste; L1 = 2 cm x 0.8 mm ID Teflon tubing; L2 – 12 cm x 0.3 mm ID tubing.

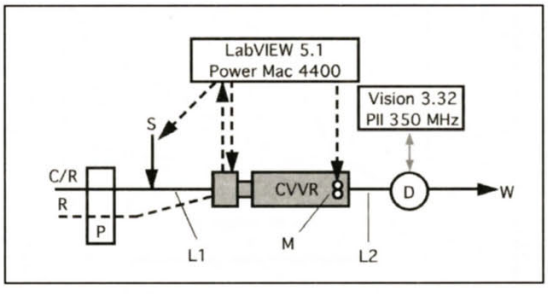


Figure 3. Continuously vairable volume housing (chamber housing made from polypropylene constructed in-house). Length 14", height 6", depth 4.25". Linear motor (#46341-12), rotary motor (#36440-12), and drive cards (#39105) from Haydon Switch and Instrument Co. (Waterbury, CT USA). Maximum linear motor translation 50.8 mm. Electronics and location sensors assembled in-house from supplies from Newark Electronics (Chicago, IL USA).

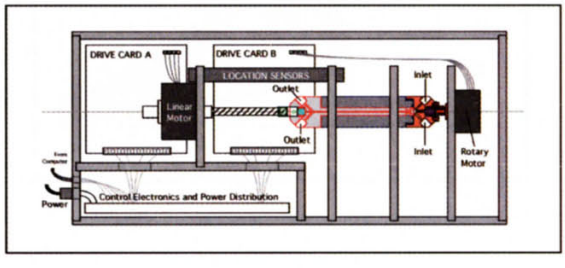
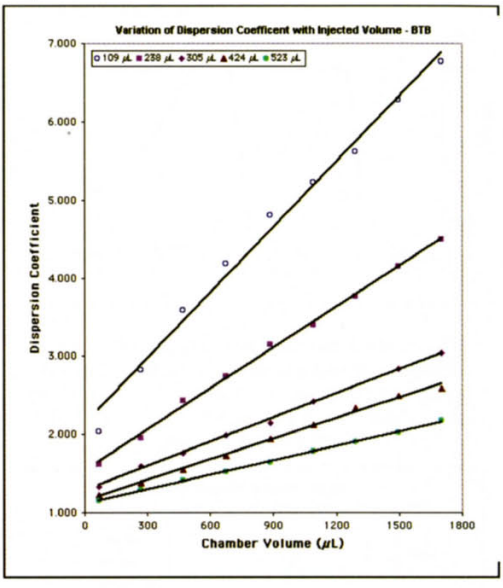


Figure 4. Variation of dispersion coefficient and peak shape with injected volume using BTB, flowrate 1.00 ml/min detection at 616 nm, chamber volume 1702 ul.

a.)



b.)

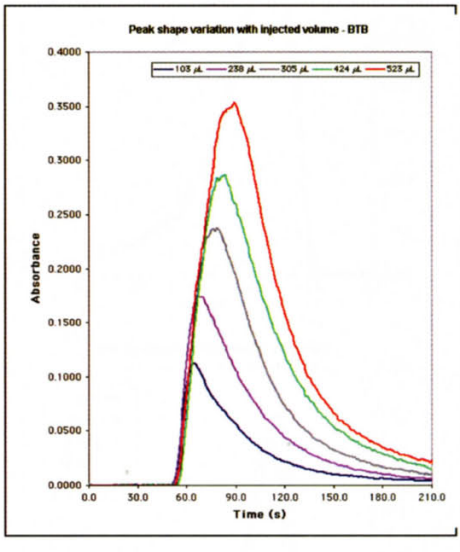


Figure 5. Variation of peak shape with flowrate using BTB, injection volume 109 uL detection at 616 nm, chamber volume 1704 uL.

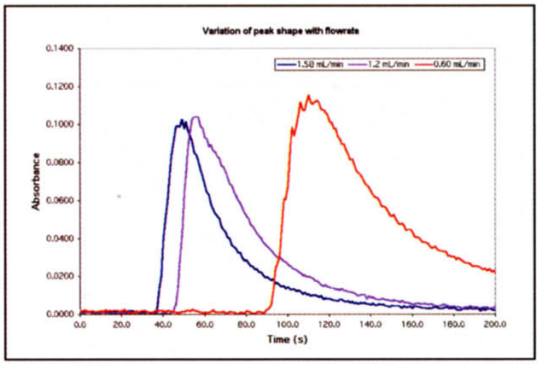


Figure 6. Variation of peak shape with rotary mixing speed and geometry using BTB, flowrate 1.00 uL/min, chamber volume 68 uL, single line manifold with carrier stream of 5 x 10^{-4} M NaOH, detection at 616 nm.

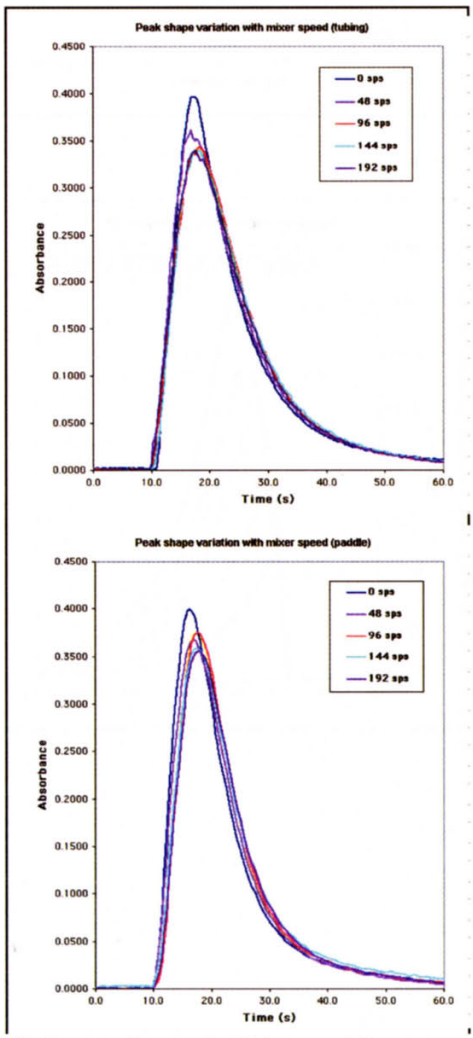


Figure 7. Variation of CVVR peak shape with injected volume 2 mg/L, flowrate 1.00 mL/min, chamber volume 885 uL, single line manifold with reagent stream of 1.55 x 10 M^{-2} 0-phenanthroline and 4 g/L ascorbic acid, detection at 512 nm.

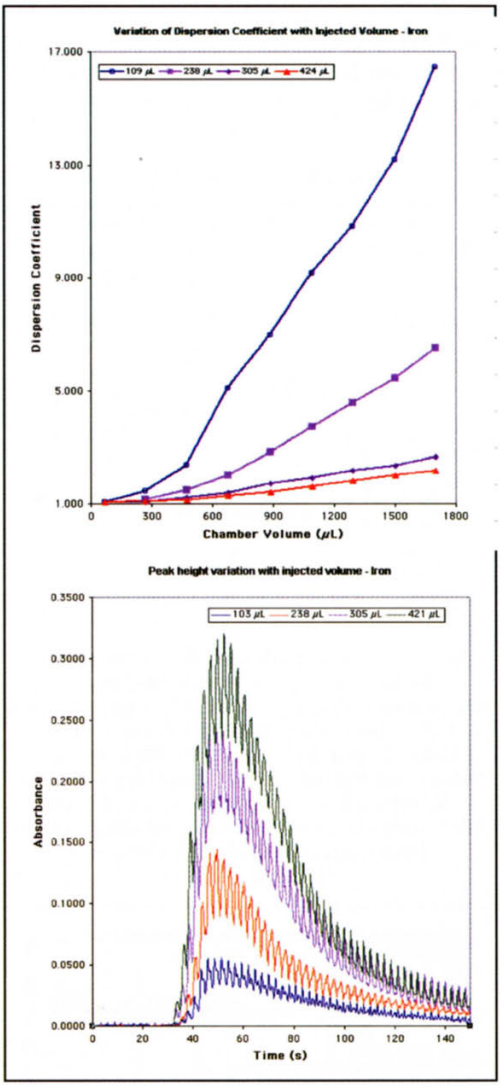
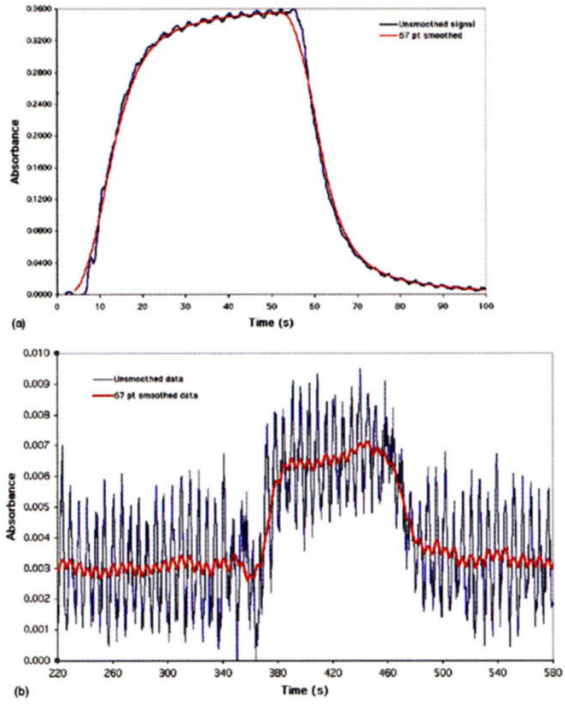


Figure 8. Smoothed and unsmoothed peaks for 2.0 and 0.02 mg/L Iron (II) flowrate 2.80 mL/min, injection volume 502 uL, double line manifold with reagent stream of 1.55 x 10 M o-phenanthroline and 4 g/L ascorbic asic, detection at 512 nm.



Dispersion Characteristics without Chemical Reaction: variation of chamber volume

A large number of experiments were performed to evaluate the variation of the dispersion coefficient (steady state signal/peak height signal) with the size of the chamber. All experiments were single line, i.e. one stream flowing into the chamber (the other inlet was plugged). In all cases the variation of the dispersion coefficient was equivalent to that produced in conventional FI manifolds.

Variation of injected volume

Varying the injected volume varies the amount of analyte injected into an FI system (using the same concentration). Thus, as you increase the volume there has to be an increase in the peak height as, for the same chamber volume, the sample is diluted less.. At each injected volume there is a linear variation in the dispersion at peak maximum due to the same injected volume getting diluted in a larger and larger chamber volume. The peak for different injection volumes show the typical FIA increase in height and width.

Variation of flowrate

Figure 5 shows that variation of the peak shape of injections of bromothymol blue (BTB) into the CVVR at a flowrate of 1.00 mL/min and a chamber volume of 1704 µL. As expected the slower the flowrate the less the dispersion and thus the higher the peak height. However, at the faster flowrates there is not a significant difference in the peak heights, something that was seen for all volumes of the chamber. This suggests that the laminar flow contribution to the overall peak shape is not as significant at high flowrates as it is at low flowrates, and therefore at this point peak shape variations are primarily due to the diffusion of the BTB.

The use of a computer to control and time the injections made it easy to evaluate the peak maximum time for each set of peaks. As the peaks appear sooner at faster flowrates a plot of flowrate (mL/min) versus the reciprocal of the peak maximum time (1/min) gave a straight line (R2 = 0.99965) with a slope of 0.826 mL^{-1} . Variation of mixer speed and geometry. The evaluation of the dispersion characteristics when the chamber was converted into a well stirred mixing chamber showed some interesting results. Figure 1c shows four different mixer "geometries" manufactured either in our laboratory or by PlasmaTech. Two of them, "Hanukah" (A) and "Y" (B) were broken during experiments and so no data is available. The other two geometries, "paddle" (C) and "tubing" (D) were both tested at different speeds and with different volumes of the chamber. Figure 96 shows examples of peaks produced for each

geometry at different speeds with the chamber fully closed. It can be seen that the peak shapes are remarkably similar for both geometries and at all speeds. Even so, and as would be expected, there is an increase with dispersion with an increase in speed of the mixer, yet the peaks are not much wider. The conclusion that can be drawn from this is that when the mixer is not spinning, the chamber acts more like a well stirred mixing chamber than a conventional mixing coil in producing more exponential peaks.

In addition, there is a speed of rotation above which the peak shape does not change significantly and this is the point where the solution is intimately mixed before it leaves the chamber and no further mixing (additional increase in speed) is necessary. At bigger volumes of the chamber (817 and 1634 µL – data not shown) reproducibility was found to be poor (>5%) at slower rotation speeds. This suggests that the mixer is producing turbulence in the solution in the chamber but not making the solution homogeneous by the time it leaves the chamber. Reproducibility got better again at higher speeds.

For subsequent work we decided to use the paddle geometry as the noise on the peaks was lower than the tubing geometry, and the washout of the chamber was slightly faster (less exponential peak). As experiments needed to be done over the all the volumes of the chamber the mixer was run at 240 steps per second (300 rpm) which was the fastest speed that rotary motor could reliably handle.

Dispersion Characteristics with Chemical Reaction

The determination of iron(II) is an excellent chemical system to study the dispersion of the CVVR because, the system has fast kinetics and a stable product (over a wide pH and temperature range), and therefore any variation seen is due to mixing phenomena and not reaction. In addition, using standards made from iron(III) requires the addition of ascorbic acid to the carrier and reagent stream. This and the high concentration of the ophenanthroline reagent used (1.55×10^{-2} M is at the solubility limit) makes for a system that requires good mixing in order to obtain reproducible reaction and consequently peak shape.

The graphs in Figure 7 show data obtained with the chamber without stirring and in a single line manifold configuration. It can be seen that there are significant differences between peaks produced with chemical reaction than those produced without. The dispersion coefficients for the reaction of iron(II) are over a wider range than for injections of BTB. This can rationalized based on the kinetics of reaction adding to kinetics of mixing, and the greater difficulty of mixing these solutions compared to BTB and NaOH. The reaction kinetics also show up in the non-linear nature of the dispersion coefficient variations at low chamber volumes. At higher chamber volumes the variations of the dispersion coefficient do become linear as complete reaction has already occurred and the product formed is simply being further diluted.

More startling are the shapes of the peaks produced in these experiments. The regular oscillation on top of the peak shape is due to pump pulsations and the reduction of linear velocity as the injected solution enters the chamber. Pump pulsations are a very common problem in FI systems, however the oscillations on the peak shapes are typically no where near as large as seen here. We speculate that as the solution surges into the chamber a fountain effect is created which generates a wave of reagent that passes out longitudinally down the center of the chamber, creating a turbulent flow regime. As the iron bolus is pushed into the chamber almost immediately upon injection, the waves of reagent traveling down the center of the chamber produce waves of product and this produces the oscillation on top of the normal FI shaped peak.

Experiments using the mixer at different speeds (with everything else the same) showed that the oscillation is still present but at a lower height peak-peak. This is explained by the fact that, at a high enough mixer speed, the pulsations of reagent enter the chamber but are then immediately mixed into the solution in the chamber before moving onto the detector. The use of the mixer dampens out the oscillation of the pump pulsations. We plan to look at this effect in the future especially with respect to the use of a pulse free pump called the MilliGat (Global FIA, Gig Harbor, WA).

It should also be noted that with this single line determination of iron(II), and no stirring in the chamber, the reproducibility of the peaks (using the maximum peak absorbance — unsmoothed) was acceptable (RSD ranged from 0.23-4.26% for five injections) and not significantly worse than the BTB experiments (0.14-3.65%). A similar variation of the peak shapes is seen for flowrate in the iron(II) system as for the BTB described earlier, and not surprisingly the oscillations on top of the FI peak changed frequency as the flowrate changed.

Determination of Iron(II) in Environmental Waters

As the iron(II) system produces FI peaks with oscillations in the single line mode we decided to look at the system in the double line mode. Again, oscillations were seen on the peaks produced in these experiments and so it seems that the CVVR is less tolerant of pump pulsations that conventional flow injection mixing coils.

However, given that a double line FI system can produce peaks that go to steady-state we decided that this was the best option for performing a calibration and analysis of samples. Previous work by this author [31] has shown that in fact the best approach to doing sensitive FI is using the double line approach. Many problems with refractive index effects are not present when double line manifold are used, and the sensitivity is essential the same as both normal and reverse FI systems [31]. The only caveat to this is the inherent limitation of dilution at the confluence point defined by the ratio of the two flowrates. In order to get the best sensitivity the dilution of the sample must be small and hence the flowrate of the reagent stream should be slow in comparison to the sample stream. As the flowrate of the reagent gets slower, the concentration of the reagent must increase in order to maintain the reagent excess required for the reaction to proceed to completion.

The ultimate consequence of this is that the reagent and sample mixing can limit the sensitivity if channeling or other non-homogenous mixing occurs. This problem is not seen in the CVVR when the mixer is at a high enough speed. Additionally, the steady-state peaks produced from a double line manifold should make it easy to smooth the oscillations on the peaks without the losing the signal — essentially filtering the noise, leaving behind the "DC" component.

Optimization of the peak height produced by 2 mg/L iron(II) was based on the conditions described previously [31]. The final conditions were a compromise between the size of the oscillations on the peaks, the ratio of the flowrates obtainable using the pump, and the time for the reaction to go to completion before detection. In terms of sensitivity the pump pulsations again limited how slow the reagent stream could be pumped and thus as mentioned above we are currently looking into a pulse free pump as a replacement for the peristaltic pump used here.

Figure 8 shows peaks produced by injection of 2 mg/L and 0.02 mg/L iron(II) into the double line manifold under optimum conditions. The smoothed signal was produced by processing the data file with a 67 point moving average filter (equivalent to 8 s of data). This is a large filter to apply, however it was determined (looking at residuals) that at the filtered peak maximum no distortion of the FI signal had occurred. For the 0.02 mg/L

standard the oscillations can still be seen on the signal·therefore it may be possible to optimize the filter even further.

The calibration curve shows excellent linearity and the RSD's of the standards are low in addition. Analysis of the pond water collected at UNF showed that even after two weeks, a spike of iron(III) could be recovered almost completely. The analysis of SRM 1643d was more difficult due to the nature of the sample, 0.05 M in nitric acid. At this pH the reaction between iron(II) and o-phenanthroline does not occur. Thus, in addition to ascorbic acid, the pH of the sample was increased to ~5. After compensating for the contamination added by the ascorbic acid and sodium hydroxide (Fisher certificates of analysis) a corrected concentration for total iron in the SRM was achieved. This compared favorably to the certified value.

Finally, the detection limit of the method was evaluated by determining the noise on the baseline of the injections of the 0.02 mg/L standard. Using the calibration curve this gives a detection limit concentration of 3.7 µg/L iron(II). Assuming we can reduce or eliminate the oscillations on the peaks it is feasible that this detection limit can be lowered even further.

Conclusion

The CVVR is a significant addition to a FI manifold. The ability to accurately and reproducibly setup a FI manifold with widely different mixing (and reaction) volumes allows for a single system that can be applied to a wide variety of chemistries. In addition, the full automation of a flow injection system opens up the possibility of autonomous analysis systems that can operate in remote and/or hazardous locations. Intelligent control software can be envisioned that could adapt the FI system to samples that go out of range, or that matrix match samples automatically. The use of the CVVR in FI titrations, stopped flow kinetics experiments, and user-defined concentration gradient generation all beg investigation. With the use of a pulse free pump, such the MilliGat from Global FIA, there is also the possibility of performing very low dispersion FIA because of the use of the mixer in the chamber. Finally, the possibility of variation of the chamber volume as the injected sample flows through the CVVR is an area that will be demonstrated in a forthcoming paper.

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